Aliphatic Delta-Lactones: Determination in Bovine Milk from Animals on Normal and Fat-Depressing Diets

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CERTAIN DAIRY PRODUCTS—namely dry whole milk, dried cream, evaporated milk and, anhydrous milk fat—develop a characteristic flavor during storage. This flavor is considered a defect by the dairy processor who is attempting to manufacture a beverage product. The defect, characterized as a coconut-like off-flavor, was shown to be due to the occurrence of δ-decalactone and δ-dodecalactone in the lipid phase of milk (1,2,3,). Since the initial identification of the 10- and 12-carbon δ-lactones, numerous investigators have confirmed these results and also provided evidence of additional aliphatic lactones associated with milk fat (Table I). The major lactones occurring in heated butterfat are the δ-decalactone, δ-dodecalactone, δ-tetradecalactone, and the δ-hexadecalactone (4). Of these the first two are of prime importance in development of the coconut like flavor in stored dairy products. The pleasant aroma associated with heated butter and foods cooked in butter is undoubtedly due, in part, to the formation of these lactones. Furthermore, it is probably one of the important classes of compounds which make butter one of the best-flavored baking shortenings. A patent was granted in 1958 permitting the addition of lactones to margarine for flavor improvement. (5).

The factors which control this flavor formation (2) are therefore of significance to the dairy industry as well as to the food industry using dairy products. Data thus far have indicated that the precursors are the 5-hydroxy alkanoic acids in esterified glyceride form (2,4,6). Hydrolysis and lactonization of the hydroxy acids yield their corresponding lactones. The evidence is quite conclusive that these lactones are formed by a nonoxidative mechanism (2). It is not known whether the common variables such as feed, season, breed, and stage of lactation have any bearing on lactone potential. The present investigation is concerned with the effects of fat-depressing diets on the levels of lactone precursors and thus of lactone flavor potential.

Materials and Methods

Three Holstein cows with similar production records and stage of lactation were selected from the University Herd; they are described in Table II. During the normal sampling period the cows received a regular herd ration and good-quality alfalfa hay. Individual milkings were collected and pooled for each cow. After this sampling period the regular herd ration was substituted gradually with a complete mix containing alfalfa hay, heat-treated corn, soybean oil meal, and the necessary vitamin and mineral supplement. The basic change in the diet was the substitution of ground corn, oats, and wheat bran in the regular ration with heat-treated corn and chopped alfalfa. The experimental ration was properly balanced for the milk production levels of the three cows. After the period of adjustment, milk samples were collected as previously described. Eight milkings from each animal on the two diets were pooled and the milk was separated immediately. The resulting cream was churned in a laboratory churn and the butter liquefied and reseparated to obtain the clear butter oil. Lots of butter oil (500 g.) from each cow on normal and heated corn diet were then steam-deodorized, with the apparatus and procedures of Tharp and Patton (3). The fat was distilled at 180°C. and pressures of 0.1–2 mm. Hg for 3 hr. The conditions were held constant for all lots of butterfat. The resulting distillate was extracted with redistilled ethyl ether. The ethyl ether extract was evaporated under N_2 on a steam bath to 5-ml. volumes.

Gas-chromatographic analysis was used for quantitation of the lactones present in the 5 ml. extract residue from the steam distillate. Analyses were made on a Bar-

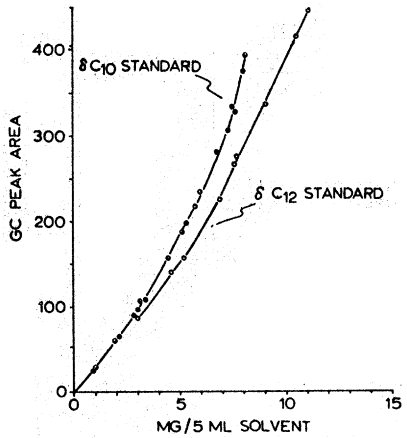

Fig. 1. Calibration curves for the concentration of δ-C10 and δ-C12 aliphatic lactone standards vs. GC peak area.

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Table I. Saturated Aliphatic Lactones in Butterfat

Lactone	*Author(s)*
δC6	Parliament *et al.* (10)
δC8	Boldingh and Taylor (11)
γC10	Jurriens and Oele (6)
δC10	Keeney and Patton (1)
γC11	Jurriens and Oele (6)
δC11	Jurriens and Oele (6)
γC12	Jurriens and Oele (6)
δC12	Tharp and Patton (3)
γC13	Jurriens and Oele (6)
δC13	Jurriens and Oele (6)
γC14	Jurriens and Oele (6)
δC14	Boldingh and Taylor (11)
γC15	Jurriens and Oele (6)
δC15	Jurriens and Oele (6)
γC16	Jurriens and Oele (6)
δC16	Boldingh and Taylor (11)

ber-Coleman Model 10 Gas Chromatograph (GC) equipped with a radium 226 detector source. Conditions were as follows: 6 ft. × 6 mm., heavy-wall, U-tube glass column packed with 60/80-mesh Gas Chrom A coated with 10% (w./w.) diethyleneglycol adipate, treated with 2% (w./w.) phosphoric acid; column temperature, 182°C.; detector temperature, 225°C.; flash heater, 265°C.; argon pressure, 16 p.s.i.g.; 750 v. applied to detector cell; and electrometer sensitivity of 10 (1×10^{-7} amperes). Individual lactone peaks were identified by comparison of retention times with reference lactones and characteristic odor of the effluent from a heated outlet from the column.

Authentic lactones, used as standards in the quantitation, readily polymerize and cause excess tailing of the GC peaks. It was therefore necessary to depolymerize the authentic lactones before GC standards were prepared. Approximately 3 g. of lactone was refluxed for 30 min. in slight excess of 7.8% KOH in ethanol. The ethanol was evaporated with the aid of a stream of N_2. The resulting soap was acidified with 1*N* HCl and the mixture warmed to decompose the soaps. This solution was then saturated with salt and cooled, and the lactones were extracted with ethyl ether. The freshly depolymerized lactone was made up to known concentrations in 5 ml. hexane. Five-microliter amounts were injected onto the GC column in each analysis. Figure 1 illustrates the concentrations of known standards *vs.* GC peak areas, which were used as a basis to quantitate the lactones in the unknown extracts.

Results and Conclusions

When the cows were placed on the heated corn diet there was a marked drop in fat yield (Table III). The average milk yield did not vary substantially with the change in diet. Table IV presents the data on the δ-decalactone and δ-dodecalactone concentrations (in p.p.m.) in the fats obtained from animals on normal and heated corn diets. A rather uniform lowering in lactone concentration resulted from the heated corn diet which amounted to approximately 25% decrease. In addition, the concentration of δ-octalactone and δ-tetradecalactone decreased similarily (data not presented).

To state the direct cause for the decrease in lactone potential would be presumptive at this time, because of the lack of complete understanding of lipid metabolism of animals on depressing fat diets. It is possible to speculate, however, that the decrease in lactone potential is a result of a deficit in lactone precursor due to altered lipid metabolism accompanying the depression in fat yield. Recently Warner (7) and Van Soest (8) reviewed the subject of fat depression, and a recent paper from this laboratory also points out the complexity of the problem (9).

It is evident, however, that the diet of the bovine does influence the lactone potential of the butterfat. This is also evident by the difference in lactone concentrations from animals on pasture during the summer months, and on roughage feed during the winter months. The concentration of lactones in a blended fat sample taken in the summer is higher than that of a blended fat sample taken in the winter (Table V). A more detailed study is under way to determine the lactone potential throughout the year.

Table II. Description of Animals Used in This Study

Cow No.	*Breed*	*Age Years*	*Time in Lactation Days*	*Av. Daily Milk Yield lb.*	*Last Lactation, Av. Fat Test %*
1026	Holstein	5	125	70	4.3
1030	Holstein	4	116	70	3.5
1060	Holstein	4	131	68	4.0

Table III. Average Milk Production and Fat Test per Milking of Holstein Cows When on Normal and Heated Corn Diet

Diet	*No. of Milkings*	*Cow 1026*			*Cow 1050*			*Cow 1060*		
		Fat Test %	*Milk Yield* lb.	*Fat Yield* lb.	*Fat Test* %	*Milk Yield* lb.	*Fat Yield* lb.	*Fat Test* %	*Milk Yield* lb.	*Fat Yield* lb.
Normal	8	3.5	28.6	1.007	3.4	27.9	0.945	4.1	31.2	1.262
Heated corn	8	1.9	31.5	0.588	2.9	28.9	0.826	3.2	27.1	0.863

Table IV. Concentration of Delta-C10 and Delta-C12 Aliphatic Lactones in Fat from Individual Holsteins on Normal and Heated Corn Diets

Diet	*Concentration* *Cow 1026*		*Cow 1050*		*Cow 1060*	
	δ C10 p.p.m.	*δ C12* p.p.m.	*δ C10* p.p.m.	*δ C12* p.p.m.	*δ C10* p.p.m.	*δ C12* p.p.m.
Normal	12.6	25.5	16.0	27.9	12.1	20.8
Heated corn	9.3	20.0	11.7	21.9	9.2	16.1

Table V. Concentration of Delta-C10 and Delta C12 Alipathic Lactone from Samples of Summer (July) and Winter (February) Blended Milk Fat

Lactone	*Concentration* *Summer* p.p.m.	*Winter* p.p.m.
Delta-C10	12.5	8.4
Delta-C12	23.7	15.6

The mode of formation of the lactone precursors and their relation to lipid metabolism has challenged practical implications. The purchasing preference of the consuming public is dictated largely by the flavor of the product. When we understand the metabolic origin and what factors enhance or suppress flavor compounds, for example the

lactones, we can improve the desired flavor of our products.

These findings may enable the processor, whether in the manufacture of butter, stored beverage dairy products, or baked goods, to select butterfat depending on the desirability of lactone flavor potential.

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